PRELIMINARY NOTE ON THE RESULTS OF CROSSING TWO HEMIPTEROUS SPECIES WITH REFERENCE TO THE INHERITANCE OF AN EXCLUSIVELY MALE CHARACTER AND ITS BEARING ON MODERN CHROMOSOME THEORIES.

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Interest in the studies of the chromosomes has been greatly stimulated during the past few years by the revival of the hypothesis that they are the bearers and distributors of all hereditary characters. Belief in such a fundamental significance of the chromosomes has led its firmest adherents to interpret every phase of their morphological and physiological expressions into terms of a causal nature, even to the point of claiming that definite chromosomes—such as the so-called sex-chromosomes—are the determining factors of sex and of all sex-linked characters.

On the other hand a large number of cytologists have studied the chromosomes from an entirely different point of view—believing that their morphological phases are not to be interpreted in terms of a causal nature, but like many other organs of the cell, they are the expression rather than the cause of cell activities.

These opposing interpretations can be strikingly demonstrated by a few quotations from recent papers. Morgan ('11) writes: "The experiments on *Drosophila* have led me to two principal conclusions: First, that sex-limited inheritance is explicable on the assumption that one of the material factors of a sex-limited character is carried by the same chromosomes that carry the material factor for femaleness.

"Second, that the 'association' of certain characters in inheritance is due to the proximity in the chromosomes of the chemical substances (factors) that are essential for the production of those characters" (pp. 365).

Wilson ('12) gives the stamp of his approval to Morgan's

conclusions. He says Morgan's results "bring strong support to the view that the chromosomes are such bearers of unit-factors, for the whole series of phenomena determined in *Drosophila*, complicated as they seem, become at once intelligible under the assumption that certain factors necessary for the production of the sex-limited characters are borne by the *X* chromosome; and without this assumption they are wholly mysterious" (pp. 420–1).

As opposed to these definite expressions of faith in the causal nature of the chromosomes, we may quote Child's latest repudiation of all such hypotheses. "Let us take the case of the chromosome, for example, which plays so important a part in recent biological hypothesis. What is the chromosome? If it is what many authors seem to believe, it is an autonomous being endowed with something more than human intelligence. But if we are not willing to believe this, then we must regard the chromosome as an incident or result of dynamic processes in the organism, like other morphological entities. If this is the correct view, then it is nothing ultimate or fundamental. We must analyze it into terms of the processes which have made it, and in this analysis we shall sooner or later find nothing more or less than the whole complex of processes which constitute the organism. The organism makes the chromosomes, not the chromosomes the organism" (pp. 33).

Our cytological studies have caused us to sympathize with the many investigators who have expressed skepticism of the causal nature of the chromosomes. For several years we have argued that the chromosomes in the forms we have studied show too much variability, both in their morphological and physiological expressions, to justify those theories which obviously demand a rigid compliance to a definite mode of expression. We demonstrated in 1905 that the form and relative size of the chromosomes in *Allolobophora fœtida* are inconstant and in every publication since that date we have demonstrated variability in the form, relative size and behavior of the chromosomes in every form we have studied, and we have consistently argued that such variability attacks the very foundations upon which the popular chromosome speculations of this decade have been built.

Recent experimental results have caused a marked modification of the views of some of the adherents of the more extreme chromosome hypotheses, forcing them to modify the theory of the individuality and continuity of the chromosomes so far as to admit that there must be an interchange of chromatin between individual chromosomes and that the chromosomes which emerge from synapsis are "probably not identical with the original conjugants" (Wilson, '12, pp. 422). They do not however extend this interpretation to the XY chromosomes: "the degree of union may vary in different cases, involving sometimes no fusion, as is suggested by the history of the XY pair" (Wilson, '12, pp. 417). This would seem to be an inevitable conclusion, otherwise any facts that could be assumed to be explained on the supposition of an interchange of factors between X and Y, would be inexplicable for those forms in which no Y is present.

In analyzing the results of our recent experiments we shall accept, for the sake of the argument, the above assumption that there is no interchange of material between the XY chromosomes, and also the hypothesis of male- and female-producing spermatozoa.

In 1909 Castle suggested that the Y chromosome of those forms in which this morphological element is present may be the bearer of all characters that are exclusively male. He wrote: "I would offer the suggestion that we have a mechanism suitable for the transmission of characters exclusively male in the Y element described by Wilson, the 'synaptic mate' of the X element."

It would seem possible to test the value of this suggestion by crossing two species, each having the XY chromosomes, and one of the species having an exclusively male character which is lacking in the other. These conditions are met in the two Hemipterous species *Euschistus variolarius* and *Euschistus servus*. The former has a character that is exclusively male, in the form of a distinct black spot on the male genital segment, while such a spot is entirely lacking on the male genital segment of *Euschistus servus* (Photo 1). There is no black spot on the female genital segment of either species.

During 1911 and 1912 we succeeded in crossing three species

of Hemiptera, but in this preliminary note we shall confine ourselves to discussing the cross between *Euschistus variolarius* female and *Euschistus servus* male. From this cross we were able to raise to maturity 11 males and 16 females of the F_1 generation and these fortunately mated readily and proved to be very fertile. Seven of these pairs were isolated and the offspring of each pair raised in separate cages—in many cases even the bugs from a single batch of eggs being isolated during the entire period of their development. From these seven pairs of the F_1 hybrids we raised 249 females and 204 males. These bugs were kept in the laboratory until several days after they had reached the winged stage, when the females were preserved as pinned specimens and 191 of the males were preserved in glycerine, as shown in photos 2[1] to 6.

An analysis of the maturation divisions would seem to support Castle's suggestion that the *Y* chromosome may be the bearer of exclusively male characters, for this chromosome is the only one that can be in both the so-called male-producing spermatozoa of each quartette of spermatids resulting from the two maturation divisions, assuming that these divisions occur as illustrated in the text-figure.

It would seem quite logical to conclude that if a character associated exclusively with the male sex can be inherited from the father, the factors which produce it should be in the so-called male-producing spermatozoön and if they are to be located in a given chromosome it must be the one and only chromosome that *ex hypothese* can be present in all the so-called male producing spermatozoa, otherwise the character would not be a constant feature.

If Castle's suggestion is correct, then all characters exclusively male can be inherited only through the father, as the *Y* chromosome is never in the female. If then *Euschistus variolarius* is fertilized by *Euschistus servus*—a form which lacks the spot—none of the offspring should have it, neither the F_1 nor the F_2

[1] We have photographs of all the male hybrids, but they must be reproduced by the bromide print method in order to secure an accurate reproduction of the shades of difference in the size and intensity of the spot. We hope to be able to reproduce all these photographs when we publish the detailed account of the crossing of the three species, *Euschistus variolarius*, *Euschistus servus* and *Euschistus ictericus*.

generation. *As a matter of fact, however, the spot of Euschistus variolarius is transmitted through the female—appearing in a slight degree in the males of the F_1 generation and much more intensely in the F_2 generation*—some of the offspring of this generation having the genital spot quite as conspicuous as that of their maternal male ancestors—*E. variolarius*. Such facts would seem to dispose beyond question of Castle's suggestion that the factors which produce exclusively male characters must be located in the *Y* chromosome.

First Sp. Cyte Chromosomes.	Second Sp. Cyte Chromosomes.	Sp. Tid. Chromosomes.
A B C D E F X Y	C E x y	A C E X — ♀
		A C E Y — ♂
	B D F x y	B D F X — ♀
		B D F Y — ♂

Scheme of the two maturation divisions of *Euschistus variolarius* and *Euschistus servus* based on the assumption that the first maturation division separates autosomes of maternal and paternal origin and the second division halves them. The *XY* chromosomes on the contrary being halved in the first division and separated in the second division. The relative positions of the autosomes may be changed unless definite chromosomes are always destined to the same pole, but reversing their position in this regard does not alter the end result—that the only chromosome common to both so-called male-producing spermatids is the *Y* chromosome.[1]

We may next examine the facts on the basis of the assumption that the factors which produce the male genital spot can be located in the *X* chromosomes. If this is true then the spot cannot be transmitted through the so-called male-producing spermatozoön for this spermatozoön contains no *X* chromosome.

We hoped to be able to test this by fertilizing a pure female *E. servus* by a pure *E. variolarius*, but owing to great scarcity of material in the locality where we were working we were unable to find any male *variolarius* at the time it was possible to attempt this experiment. We were, however, able to cross a pure *E. variolarius* male with an F_1 female and compare the male offspring with those obtained by the F_1 matings.

[1] We have used the method of designating univalents by the letters of the alphabet, bivalents being represented by A B, C D, E F. Only six of the twelve autosomes are designated.

If, as stated above, we attempt to locate the spot factors in the X chromosomes, we cannot expect these factors to be transmitted through the male producing spermatozoon and therefore if an F_1 female hybrid is fertilized by a pure *E. variolarius*, we should expect to have a no more pronounced spot in the offspring than we obtain by matings of two F_1 hybrids. *The facts, however, demonstrate that the spot is transmitted through the pure male E. variolarius far more intensely than it is through the F_1 male.* This is strikingly illustrated by a comparison of the male offspring of an F_1 female and pure male *variolarius* with the 191 males we have succeeded in raising from seven pairs of the F_1 hybrids. We have 18 males from the former pair and many of them have the genital spot stronger than any of the 191 males from the F_1 parents. Further, none of these 18 males are without any spot, *whereas 84 of the 191 males of the F_2 generation have no spot whatever.*

Our facts seem to have demonstrated two points: First, that the spot is inherited through the female without the aid of the Y chromosome, and second, that the spot is inherited through the male without the aid of an X chromosome. We seem forced then to admit that in this case neither of the so-called sex chromosomes is necessary to the inheritance of an exclusively male character, the factors of which, it would seem, ought to be contained in a chromosome which determines sex, if, in fact, sex determining chromosomes exist. The spot in *E. variolarius* is a part of the male genital segment and it is only logical to assume that the factors which produce it are associated with the factors which produce the male genital segment itself, wherever or whatever those factors are. As the facts do not seem to warrant the assumption that these factors are confined to either of the so-called sex-chromosomes, we may re-examine them in the light of placing these factors in some other chromosome or chromosomes.

As the so-called male-producing spermatozoön of many forms has no Y chromosome and therefore no male sex-chromosome, it is obvious that if this spermatozoön is assumed to carry a factor which produces sex, this factor must be placed in some other chromosome, by those who believe that the chromosomes

are the vehicles of such factors. Realizing this Morgan ('11) concluded that "the factors for producing the male must be located in some other chromosome" and he places these factors in a pair of homologous chromosomes, as shown by his scheme.

Gametes of female — X M—X M
Gametes of male -- X M—M.

A glance at text-fig. 1 will show that placing the male factors in an homologous pair of chromosomes (in *A* and *B* of Fig. 1 for example) necessitates their presence in all the spermatozoa of both sexes, thus giving to the so-called female-producing spermatozoön the male-producing factors in addition to the female-producing factors. The female-producing spermatozoön is therefore heterozygous for sex and the male-producing spermatozoön homozygous for sex—and this seems to involve a denial of the assumption that has been the basis of so much experimental analysis, namely, that the female is homozygous for sex. How can the female be homozygous for sex when the so-called female-producing spermatozoön has conveyed to her "the factors for producing the male"?

If we assume with Morgan that both members of a definite diploid pair of chromosomes contain the factors for producing a male, this must hold for the same diploid pair in both the female and the male, and if the factors producing exclusively male characters are linked with the factors for maleness, then each member of this diploid pair should contain the factors for producing the spot, which in *E. variolarius* is an exclusively male character.

As this diploid pair is in the female as well as in the male, it would seem necessary to assume that in *E. variolarius* the female carries an inhibitor of such exclusively male characters. But one is embarrassed in considering where to place this inhibitor—if we locate it in both members of an homologous pair of chromosomes, then it would be in the male as well as in the female. If we place it in only one member of an homologous pair (in *A*, for example, of text-fig. 1), this would involve selective fertilization, for if the female is fertilized by the so-called female-producing spermatozoön that is without the *A*, and she has by

chance discarded the other *A* in her polar body, she also would have the spot, and further, half the male producing spermatozoa have the *A* chromosome and therefore they must not function or the spot would be absent in half the variolarius males.

If we place the inhibitor in the three *X* chromosomes, the assumption obviously would not work, for the male has one of the three *X* chromosomes and the spot would therefore be inhibited in the male as well as in the female. It would therefore be necessary to assume that in *E. variolarius* two doses of inhibitor are necessary to cancel the spot. This assumption, which apparently would hold for *E. variolarius*, is, however, found to be untenable if used to explain the results of the cross between *E. variolarius* and *E. servus*, for the female hybrid cannot receive a double dose unless we assume that *E. servus* carries an inhibitor for inhibiting in the female a male character which is never present in the male. If, however, we feel justified in assuming that the female hybrid has a double dose of inhibitor, this would fail to explain the absence of the spot in those male hybrids, which do not have it, for they have only one *X* chromosome and therefore only one dose of inhibitor. We might avoid this by assuming that one dose only would be necessary for the female hybrid, as she presumably has only one dose of spot factors; but this would involve inhibiting the spot in all the F_1 males also, which is opposed to the facts, as the spot is not inhibited in all the F_1 hybrids. The facts show that the spot factors—whatever they are—are latent in the female and if they are suppressed by an inhibiting factor, it would seem that this cannot logically be located in a definite chromosome.

Our results demonstrate that the spot is by no means a unit character. If we speak of it in terms of Mendelism we must say that it is the result of a large number of unit factors, for in the 107 males of the F_2 generation in which a spot can be identified, it is present in every degree of intensity, from a mere indication of a spot to those which are nearly, if not quite, as conspicuous as the spot of a pure *variolarius* (Photos 2–4).

If we assume that each member of one diploid pair of chromosomes contains all or half the factors for the genital spot, in addition to the factors for maleness, then the ripe egg of *E.*

variolarius contains one such chromosome and it follows when the egg is fertilized by *E. servus* it will have one diploid pair of chromosomes that is homologous for the factors producing a male, but heterozygous for the factors producing the spot.

Such a diploid pair should be present in all the offspring—both males and females, and if we cancel it in the females by assuming an inhibitor somewhere, we still have it in the males and therefore the spot should be quite as pronounced in the F_1 hybrid males as in *E. variolarius*. The facts, however, are as follows: None of the 11 male hybrids have a spot as strong as *E. variolarius*, 2 have no spot whatever, 3 have a faint spot—like that of the third bug from the bottom of the tube in photo 4—4 have a slightly stronger spot, and 2 have a spot about one-third as pronounced as that of *E. variolarius*. The genital spot in the F_1 hybrids is very variable, it is not expressed as a dominant, a recessive or a true blend.

If we attempt to explain these results in terms of chromosomal distribution of the factors, we encounter serious difficulties and are forced to assume the illogical position of suggesting a method of chromosome division for the hybrids which obviously does not occur in *E. variolarius*, as the spot is not variable in this form.

Assuming that the diploid pair of chromosomes in the egg of *E. variolarius*, which was fertilized by *E. servus* is heterozygous for the spot factors (that they are in chromosome *A*, for example, of the pair *AB*), then it must be asked why those in chromosome *A* are completely suppressed in some of the F_1 hybrids, while part of them find expression in other hybrids.

We might simply assume that some of the factors of chromosome *A* have dropped out, but the facts show this to be untenable for the spot reappears in the F_2 generation—in some cases quite as pronounced as in the pure *E. variolarius*. We must thus assume that the female *variolarius* has at least half the spot factors which she transmitted to the male hybrids, although these F_1 hybrids show either no spot at all or an incomplete spot. To account for these facts we must assume that the male hybrids differ from the pure males in having an inhibitor that inhibits those spot factors which are present but which are not expressed. We have seen that the facts will not allow placing the

inhibiting factors in either the X chromosomes or the ordinary chromosomes and we are thus forced to admit that inhibiting factors—whatever they are—must be located outside the chromosomes—in the region of pure hypothesis. The facts force us to consign to these hypothetical inhibitors, not only the responsibility of suppressing the spot factors in all the females, but also of determining just how many spot factors shall find expression in the males of the F_1 and F_2 generations, and thus they practically relieve the chromosomes of the burden of unit distribution.

Chromosomes.—The small idiochromosome is an exclusively male character—a feature it has in common with the genital spot—and if it could be put to the same test of experimental breeding, which we have attempted for the genital spot—the results ought to show whether this idiochromosome has indeed the individuality which modern chromosome theories demand or whether—like the genital spot—its relative size is merely a structural feature which can be transmitted through the female. We confidently expected to be able to apply this test to the small idiochromosome of *variolarius*, for it had been demonstrated that this chromosome in *variolarius* was relatively much smaller than in *servus*.

The chromosomes of *Euschistus servus* were studied by Wilson in 1906 and he concluded that the XY chromosomes of this form differed from those of *E. variolarius* in their relative size. He says: "The above described species of *Euschistus*, while agreeing precisely in the general relations, present individual differences so marked as to show that even the species of a single genus may be distinguishable by the chromosome groups. In this case the most interesting feature is the series shown in the inequality of the idiochromosomes, which become progressively greater in the series (1) *E. servus*, (2) *tristigmus*, *fissilis*, (3) *ictericus*, (4) *variolarius*, the inequality in the last case being fully as great as *Lygæus*" (pp. 17). He demonstrates a marked difference in the relative size of the idiochromosomes of *E. variolarius* and *servus* in *a* and *c* of his Fig. 4.[1]

[1] We have a large number of photographs of entire groups of chromosomes of *E. variolarius* and *E. servus* and we have also preparations from which we can photograph the chromosome groups of the F_1 and F_2 generations of this cross from both the testes and embryonic stages, but these results are reserved for a later publication, in which we shall give a complete account of our experiments in crossing *E. variolarius* with both *E. servus* and *E. ictericus*.

Hoping that such a marked difference in the chromosomes might be a constant feature, we anticipated being able to obtain some interesting comparative results by crossing the two species. We were disappointed, however, to find that in all the individuals we studied the inequality in the size of the idiochromosomes of *E. servus* is quite as marked as in *E. variolarius*, and that the chromosomes of these two species were therefore of no special value for this comparative study.

This seems to be another case pointing to inconstancy in the morphology of the chromosomes—as the individuals which Wilson studied showed a marked feature which was absent in the individuals studied by us. All such examples of inconstancy in vital processes throw additional light on the fact that investigators of the same material so frequently hold diametrically opposed views as to the fundamental significance of such expressions of vital activities, and that facts in support of these opposing views can be demonstrated in the same material.

This is almost amusingly illustrated by two recent papers on the cytology of certain Hemiptera, Gross ('12) and McClung and Pinney ('12). Gross differs from Wilson as to the facts on almost every point in which their hypothetical views clash. Of the cytological evidence for Wilson's theories, he says, "Geleitet von einigen Gesichtspunkten, die ich bei der Untersuchung von *Syromastes* und *Pyrrhocoris* gewonnen hatte, habe ich aus dem Studium der Literatur den Eindruck erhalten, das die scheinbar so sichere cytologische Basis von Wilson's Theorie hochst unzuverlässig ist. Ja, man kann mit gutem Grunde sagen, sie existiert eigentlich gar nicht."

He denounces Wilson's work on *Syromastes* as follows:

"Im einzelnen enthält Wilson's recht kursorische Darstellung zahlreiche, leicht nachzuweisende Lücken, Ungenauigkeiten und Irrtümer."

McClung ('12) condemns our work on *Anasa tristis* with quite as firm a hand, attributing to our methods of technique our failure to demonstrate the facts which he considers of most value. With the finality of a judge he hands down his decision: "It is my judgment that this method '*used alone*' is entirely inadequate for accurate results, and in this particular case is

responsible for the discrepancy between these investigators and Paulmier, Wilson and Montgomery."[1]

A few facts will serve to convince any unprejudiced cytologist that our method of technique should not be condemned as "inadequate." From many of our preparations it is possible to demonstrate a large number of chromosome groups in which every chromosome is present and can be clearly photographed—in one case we have more than 150 photographs of such groups from the *same embryo*. A method that makes such results possible can hardly be condemned as "inadequate" for "accurate results"—it is a method, rather, that compels a recognition of enough variability in the same individual to make one very cautious in accepting premature hypotheses based on insufficient data.

In further condemnation of our methods McClung criticizes the value of photography as a means of demonstration. He says: "Foot and Strobell have employed photography alone as a means of presenting illustrations of their material, and it is assumed by them that if a thing can be photographed it must necessarily be a true picture of normal conditions. This I consider to be a decided fallacy. A photograph is an interpretation by the observer, just as is a drawing. The personal factor is no more absent from one method of illustration than it is from the other. Photographs may present with greater fidelity the details of structure in an object, but the choice of the object, and the nature of details are at the command of the photographer" (pp. 369).

We certainly do *not* assume that a photograph must of necessity represent only normal conditions, but we do believe that abnormalities are likely to be due to a pathological condition of the cells and that such a condition can quite unconsciously be obscured in a drawing, but not in a photograph. McClung's position is certainly unique when he claims that the personal factor is no more absent from a photograph than from a drawing and he bases this on the fact that an investigator may in either case *select* the object he wishes to photograph.

[1] It is difficult to understand why McClung mentions and underscores "*used alone*" as on the same page he quotes from our paper in which we distinctly state that we use sections also for "comparative work."

It may be said that in every paper representing cytological research one or more hypotheses are on trial and it is certainly the privilege of the investigator to select for illustration such evidence in his preparations as support his own convictions in regard to the hypothreses under consideration.

Our skepticism of the sex-determination theory led us to study our preparations of *Anasa tristis* with a view to determining whether the phenomena involved in the theory were sufficiently constant to justify it. We found enough evidence to fully confirm our skepticism and we claim that we had the right to select that evidence from our preparations and give it, with as much emphasis as possible.

The facts are simple and clear. We demonstrated that in *Anasa tristis* the reverse of the facts demanded by the sex-determination theory were clearly present and could be illustrated by photographs. The interesting feature in these opposing observations is the fact that we do not deny the observations of our opponents, whereas their belief in the causal nature of the chromosomes compels them to set our observations aside or to resort to strained explanations in order to account for them.

It is the old story so familiar to cytologists—if a feature is where, hypothetically, it ought not to be it is an artifact, and if it is not where it ought to be, it is due to faulty technique.

We believe that the photographs of our preparations prove conclusively that the accessory chromosome in *Anasa tristis* does not always fail to divide in the second spindle and the case may safely rest on a comparison of McClung's photographs of his preparation with ours.

We claim that the facts we have demonstrated in *Anasa tristis* are at least worthy to be placed in evidence against the chromosome theories that necessitate such facts being thrown aside as artifacts or as expressions of a pathological condition. We hold that they have at least the same value as normal variations in other organs of the cell and that they should be accepted as the same type of evidence we gave in '05 against the theories based on definite forms of the chromosomes. We demonstrated a degree of inconstancy in the form of the chromosomes of *Allolobophora fœtida* that should not exist if the hypotheses were valid.

It is interesting that such an experienced student of the cytology of the Hemiptera as Gross ('12) should also find that there are facts in *Anasa tristis* that are out of harmony with the theories. He writes:

"Später bin ich dann durch die Freundlichkeit der Herrn Dr. E. R. Downing noch in den Besitz einiger Präparate von *Anasa tristis* gelangt, deren Studium mir gleichfalls zeigte, das Wilson's Theorie mit den Tatsachen schwer vereinbar ist."

Nucleolus.—In the few Hemiptera we have studied we have interpreted the chromatin nucleolus of the first spermatocyte as the homologue of the nucleolus of other forms.

This is due to the fact that we have failed to find any other structure in the cell which we have felt justified in interpreting as a nucleolus. We were wrong in interpreting the chromatin nucleolus in *Anasa tristis* as independent of the accessory chromosome, an interpretation we were first led to doubt in our study of *E. variolarius* '09, and we stated in giving our results in this form that we would reserve the publication of the evidence until we could control it by a comparison with other forms. Buchner's work ('09) on the accessory chromosome enabled us to harmonize our conflicting evidence on this point. We wrote: "In this paper Buchner's observations on the accessory chromosome appear to throw some light on certain conflicting facts observed by us in *Euschistus variolarius*. Buchner supports Wassilieff in observing that only part of the substance of the chromatin nucleolus gives rise to the accessory chromosome. This would seem to indicate that the chromosomes in question are evolved from a nucleolar mass of chromatin, thus homologizing this structure with the cases in which it is claimed all the chromosomes are evolved from a large nucleolus, leaving a nucleolar residue after the chromosomes are formed. In *Euschistus* we find cases in which both the idiochromosomes and a chromatin nucleolus are present at the same time. Such facts, added to those cases in which the size relations of the chromatin nucleolus do not agree with those of the idiochromosomes, raise the question as to the identity of the two structures, though these facts would not conflict with homologizing the chromatin nucleolus with the nucleolus, which in some forms is said to give rise to all the chromosomes."

If these investigators are right in claiming that the chromatin nucleolus is in fact a chromosome which retains its individuality and continuity through the growth period, then it is quite necessary that another structure should be found in these cells which can be homologized with the nucleolus of other forms. If one is obviously not present, some plausible explanation of its absence must be given. If it is not figured in drawings, then the observer can be accused of superficial and careless observation; but if it is absent in photographs, then the old scapegoat—faulty technique—is held responsible.

This is McClung's explanation of the fact that a second nucleolus is conspicuously absent from all our photographs of the growth period of the spermatocytes of *Anasa tristis.* He says: "Owing to the technique employed by them the plasmosome is practically destroyed." It is certainly highly improbable that a method that has not destroyed the plasmosome in the other forms we have studied should destroy this structure in these Hemiptera.

It is interesting to notice how the plasmosome of *Anasa tristis* has developed and waxed strong since the earlier investigators described and figured it. It was described as a pale body and figured accordingly, but in Pinney's recent drawings it has developed into a strong dense body fully as chromatic as the chromatin nucleolus. Although Wilson himself describes it as a "*pale*" body, McClung's photographs from Wilson's preparations show that the structure which McClung interprets as a plasmosome is not a pale body, but is fully as chromatic as the chromatin nucleolus. Here, as in other points where our conclusions are condemned, we are willing to rest the case on a comparison of McClung's photographs with ours.

Our belief that the chromatin nucleolus of the Hemiptera is the homologue of those nucleoli of other forms that are said to give rise to all the chromosomes has been greatly strengthened by our study of the spermatogenesis of *Euschistus crassus.* In this form there are two chromatin nucleoli, one of which gives rise to the idiochromosomes and the second gives rise to a pair of autosomes—the so-called ordinary chromosomes.[1]

[1] These results were sent to press last February.

All such deviations from a method of development that has been claimed to be sufficiently rigid to justify far-reaching generalizations are a warning against formulating general laws that do not take into account all the facts.

If our results demonstrated in *Anasa tristis* be shorn of all their significance except their admission as normal variations, they still stand as a protest against premature generalizing. We expressed this in an earlier paper (1911) in reference to our demonstration that the accessory chromosome in *Anasa tristis* does not always fail to divide in the second spindle. "If the significance holds which has been attributed to the failure of this chromosome to divide in one of the two maturation divisions, then those cases in which it divides twice in *Anasa* must be set aside as pathological, the spermatozoa resulting from these divisions having no functional activity. Only to those who have endowed the chromosomes with causal attributes can variations in their behavior cause embarrassment. If we find them subject to marked variations it is a characteristic they have in common with other structures in the cell—the nucleolus, the centrosome, the mitochondria, the polar rings and other structures which have been found to be so variable that interest in speculations as to their possible causal significance has steadily waned, and those who believe the chromosomes are equally variable may justly suspect that the hypotheses surrounding these structures may be destined to the same fate as the speculations so long surrounding other cell organs, notably the centrosome."

Nearly all cytologists agree that experimental breeding has been and may ever be the most trustworthy test of hypotheses which are based on the morphology of the cell and which claim to offer a mechanical explanation of heredity.

The chromosomes of the Hemiptera are largely responsible for the modern chromosome hypotheses, for certain stages in their growth and development which are assumed to justify these hypotheses can be clearly followed and demonstrated. Those Hemiptera therefore which show the chromosome phenomena necessary to a given hypothesis are especially fitted to test its value by experimental breeding.

It would seem that a cross between two species of these Hemiptera, one of which contains an exclusively male character which is absent in the other, should furnish some trustworthy evidence for or against the hypothesis that the factors for determining sex are located in a definite chromosome of the so-called male and female-producing spermatozoa.

We believe that our results from this experimental work are in harmony with our cytological evidence on this point and that these results support the skepticism of those investigators who believe that the evidence given by the chromosomes does not warrant the assumptions that have endowed these cell structures with causal attributes.

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Description of Plates.

In all cases only the ventral surface of the bugs is shown. The specimens in photos 2 to 6 are preserved in glycerine. The genital segment of each bug has been pulled out and cotton inserted behind the segment to hold it in position to show the entire ventral surface. The bugs are magnified about 1½ diameters.

The photographs are reproduced by the half-tone method which, unfortunately, is not entirely satisfactory for showing the delicate shades of difference in the intensity of the spot.

Photo 1. *Euschistus variolarius* male, showing the ventral surface and the clearly defined black spot always present on the genital segment of the males of this species.

Euschistus servus male, showing ventral surface and the genital segment without any trace of the black spot characteristic of *E. variolarius* males.

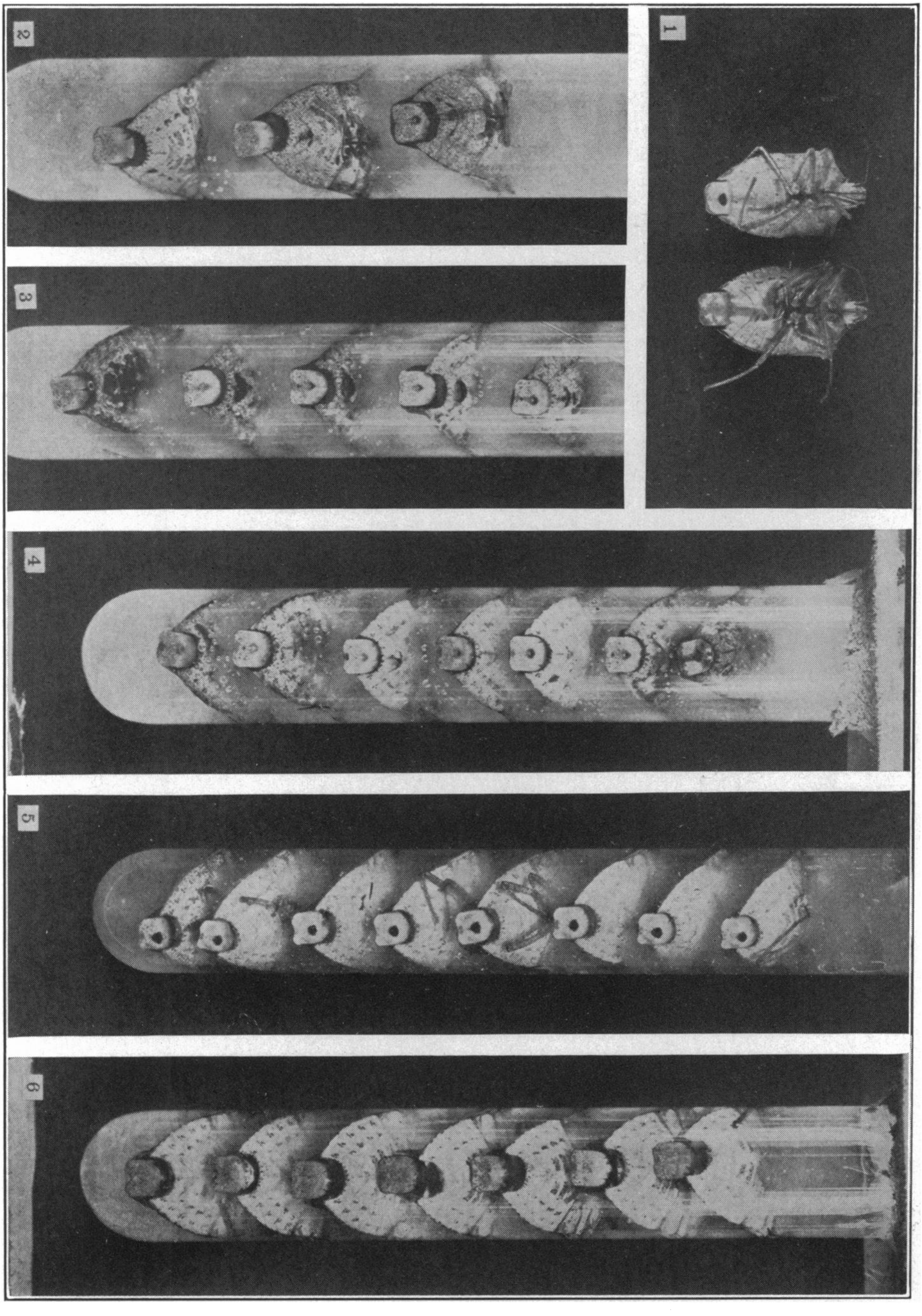
Photos 2 and 3. 8 of 46 males of the F_2 generation raised from a pair of F_1 hybrids, which were bred from an *E. variolarius* female raised in our laboratory, from a pair collected in Connecticut, and an *E. servus* male collected in North Carolina. Four of these F_2 individuals have no black spot on the genital segment, and four show the spot in a greater or less degree.

Photo 4. 7 of 54 males of the F_2 generation raised from a pair of F_1 hybrids, which were bred from an *E. variolarius* female collected in Connecticut and an *E. servus* male collected in North Carolina.

These F_2 individuals show the variations, from no spot on the genital segment, to one almost as pronounced as that of a pure *variolarius*.

Photo 5. *Euschistus variolarius* males, showing the typical black spot on the genital segment.

Photo 6. *Euschistus servus* males, showing the genital segments without the spot characteristic of *E. variolarius*.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ERRATUM.

Substitute this for the table on p. 191, Vol. XXIV.; paste in place.